



## ORIGINAL ARTICLE

# Effect of sprouting temperature on selected properties of wheat flour and direct expanded extrudates

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**Abstract**

The effect of the sprouting temperature on wheat sprouting and on the properties of expanded extrudates produced from sprouted wheat flour was studied. Therefore, wheat was sprouted at five different temperatures and subsequently dried, milled, and the resulting flour was used to produce extrudates using a twin-screw extruder. In order to understand the sprouting temperature effect, the degree of sprouting (DoS) of the differently sprouted samples and characteristic properties of flour and extrudates were studied and compared. During sprouting of wheat with increasing temperature and time an increase of the  $\alpha$ -amylase activity, the vitamin C and reducing sugar content, and a decrease of the peak viscosity was observed. The greatest effect was found at 20°C. Furthermore, the lowering of the viscosity of the flour suspension results in a reduction of the pressure and temperature in the extruder die. The extrudates of sprouted wheat flour were found to be easier to break, had a lower density, an increased longitudinal expansion index, and an improved cold-water solubility. A good correlation between the DoS and other properties of flour and extrudates was found, indicating a good predictive power and applicability of the DoS concept for wheat samples and their product development and specification.

**Practical Application**

The use of sprouted wheat flour for the production of extruded, direct expanded breakfast cereals is a promising opportunity to alter extrudate properties. Thereby, the sprouting temperature can be used as a means affecting the sprouted grain and extrudate properties intentionally and developing products being crunchier, and having an improved cold-water solubility, a lower density, and a changed expansion behavior. Moreover, due to an increased amount of reducing sugars in sprouted flour, which is a result of an intense starch degradation during sprouting, less additional sugar is needed to produce sweet breakfast extrudates.

## 1 | INTRODUCTION

Sprouting processes have been widely used in order to improve the nutritional quality and flavor of food products as well as to optimize the food

production process. Sprouted grains have already been used for years in the malt production because of its boosted enzyme activity, which is crucial to produce beer. Especially barley is steeped and sprouted for around 6 days at 14°C and subsequently kiln-dried (Jacob, 2016).

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During the sprouting process  $\alpha$ -amylase is one of the most important enzymes because of its starch degrading activity. Thereby,  $\alpha$ -amylase hydrolyses the  $\alpha$ -1,4-glycosidic bonds degrading starch into dextrins, maltose, and glucose. The metabolites except glucose are further degraded by  $\beta$ -amylase and limit dextrinase to glucose (Bewley, 2001).

According to various studies sprouted grains have an increased amount of essential amino acids (van Hung, Maeda, Yamamoto, & Morita, 2012), more vitamins (Harmuth-Hoene, Bognar, Kornemann, & Diehl, 1987), and free phenolics (van Hung et al., 2012), an increased bio-accessibility of minerals (Platel, Eipeson, & Srinivasan, 2010) and a decreased level of phytic acids (Harmuth-Hoene et al., 1987).

Because of these advantages sprouted grains could be a means to produce superior extruded breakfast cereals. Usually breakfast cereals are produced on the basis of grain flours, water, sugar, and additives like cacao or baking agents. Typical properties of extruded, direct expanded breakfast cereals are their puffed and crunchy texture, a low density and a long stability in milk or water, which has been correlated to a low cold-water solubility index.

Sprouting occurs at a moisture content of at least 30%, at adequate temperatures in the range of 10–30°C and under aerobic conditions. Thus, the biological processes in the grain during sprouting can be controlled by varying these conditions (Narziss & Back, 2012).

An adequate sprouting temperature is discussed to improve the nutritional value of the grains and to increase the enzyme activity, and, therefore, also the starch degradation. But sprouting will also increase respiration losses. Temperature and time are two of the main factors also determining the growth of microorganisms. For example, microorganism growth is doubled at a temperature increase of 10 K until the maximum temperature is reached at which microorganisms can survive. Dziki, Gawlik-Dziki, Kordowska-Wiater, and Doman-Pytka (2015) showed that the microorganism level was within an acceptable limit by sprouting processes lasting up to 4 days at 15 or 20°C. At higher temperatures or longer sprouting times the aerobic bacteria and mold growth were enhanced to unacceptable levels. Molds were found after 4 days when sprouting took place at 25°C and after 8 days when samples were sprouted at 15°C.

Finding an optimal sprouting temperature in regards to different objectives is of great importance and was part of various research studies. The alteration of the typical sprouting temperature of 14°C in the barley malt production can result in a faster sprouting process accompanying with lower production costs. For example, Müller and Methner (2015) developed an optimized barley malting process. By increasing the sprouting temperature from 16 to 24°C a reduction of the sprouting time by 48 hr (24 hr per 4°C) could be established. In addition, the increase of the sprouting temperature also results in a reduction of the energy consumption because of a lower cooling demand (Müller, 2015).

In earlier work, the degree of sprouting (DoS) was introduced and verified to be a simple characterization of the sprouting progress (Krapf, Kandzia, Brühan, Walther, & Flöter, 2019). A fast, visual evaluation of the sprouted grains allows the classification into different degrees of sprouting and the subsequent prediction of sprouted grain

and extrudate properties such as vitamin C and reducing sugar contents as well as hardness and expansion of extrudates produced from these sprouted grains. Thereby, the applicability of the DoS was already shown for oat grains (Krapf, Kandzia, et al., 2019). Moreover, the influence of sprouting time on the properties of extrudates produced from flour of sprouted wheat grains was studied by sprouting wheat for different sprouting durations at 20°C. These sprouted wheat grains were milled and the resulting flours were used for the production of extrudates. Studying the properties of the extrudates allowed their correlation with the DoS (Krapf, Arysanto, Walther, & Flöter, 2019).

In this work, the previous study was further extended and continued. Thereby the aim of this work was to study the variation of the sprouting temperature and its effect on flour and extrudate properties. The so-called DoS was evaluated for various sprouting temperatures and the results were analyzed for the prediction of the properties of extrudates from sprouted wheat grains. Therefore, wheat was sprouted at five different temperatures up to 3 days and the DoS of the samples was determined. Additionally, the DoS was correlated with other characteristic grain properties such as  $\alpha$ -amylase activity, peak viscosity of the flour suspension, reducing sugar and ascorbic acid (vitamin C) content. Finally, flours from the differently sprouted grains were used to produce extrudates. The effect of the sprouting temperature on the extrudate properties and the relation between DoS and extrudate properties was studied. Extrudate properties considered in this study are expansion, density, hardness, and the cold-water solubility index. Additionally, the effect of the sprouting time that was studied previously (Krapf, Arysanto, et al., 2019) and the temperature effect on extrudate properties were compared to be presented in a more comprehensive way.

## 2 | MATERIALS AND METHODS

The soft wheat variety *Runal* was used throughout the study (59.3% total starch, 14.1% protein, 13.4% fiber). The grains were stored in sealable containers at around 10°C prior to the study.

### 2.1 | Sprouting process

For the first part of the study investigating the temperature and time effects on wheat sprouting, 500 g of wheat grains were washed for 30 min under running tap water to clean the grain surface from microorganism and to avoid their later growth. Then, the grains were steeped in a closed container and covered with water: 4.5 hr wet steeping, 19 hr dry rest, and 4 hr wet steeping, all at 20°C. This steeping time was determined in preliminary tests and is in accordance with the recommendations of the Central European Commission for Brewing Analysis (MEBAK) (Jacob, 2016). After the steeping step the grains were dripped and put on a metal sheet which was part of the sprouting step. The steeped grains were covered with cling film to prevent the loss of water and were put in a climate cabinet (Lovibond 220 P-02) in the dark.

During this sprouting step, the grains were washed once a day using a sieve to clean the grains and prevent drying. This experimental procedure was performed at five different temperatures (10, 14, 20, 25, and 30°C) for three different periods (1, 2, and 3 days). A temperature of 14°C is usually used in the malting step for brewery purposes (Jacob, 2016). At the end of the different sprouting periods, the grains were directly deep-frozen and subsequently freeze-dried (Beta 1-16—Christ) at −40°C and 0.05 mbar for around 60 hr until a final moisture content in the range of 4–8% (w/w) was reached. Before analysis the wheat was ground in a speed rotor mill (Pulverisette 14—Fritsch) with a sieve ring of 0.5 mm at 8000 rpm.

For the extrusion experiments a large amount (3 kg) of sprouted grains was needed. For this purpose, the wheat grains were again sprouted at five different temperatures (10, 14, 20, 25, and 30°C) for 3 days. For these samples, the steeping and sprouting process was conducted in a self-constructed sprouting box using the climate cabinet of Lovibond 220 P-02 which was aerated permanently with conditioned air, humidified at a constant temperature of 20°C. The same steeping regime as explained above was applied. After the sprouting process of 3 days the grains were dried in the malting plant A1-2008 from Seeger at 65°C until a final dry matter content of 88–90% (w/w) was reached. This level of drying was chosen such that microbiological stability and millability were ensured. The parameters applied were determined in preliminary tests. The dried, cooled sprouted grains were milled using a hammer mill (Siemens-Schuckertwerke AG) with a mesh size of 500 µm.

## 2.2 | Extrusion process

Extruded products were manufactured utilizing a Berstorff ZE 25 extruder with a screw length of 870 mm at standard settings (screw speed 200 rpm, throughput 6 kg/hr, die hole diameter 3 mm). Flour with a known moisture content was mixed with tap water to achieve a water content of 27%. These settings resulted in a maximum sectional expansion index (SEI) in preliminary tests. Mixtures of sprouted flour and water were used exclusively as feedstock in order to emphasize on the differences between the different sprouted wheat flours.

The chosen temperature profile over the different barrel elements of the extruder was 20–45–65–85–120–140–140–140°C. The screw was assembled of standard forward elements with two different pitches. Once the extrusion process was stabilized the pressure and temperature in the die were experimentally determined 10 times per second. The mean pressure and die temperature per setting were calculated from datasets of at least 1,000 individual data points.

The produced extruded strands were cut into pieces of about 0.5 m length after leaving the die. They were dried at 65°C for 1.5 hr in an oven (Thermo Scientific T 6420). The expansion index, the texture measurement and the density were determined directly on the extrudates. The cold-water solubility index, the total starch and the sugar content were determined using milled extrudates. Extrudates were milled by exposing them first for 30 s to a kitchen blender (Petra MZ12.35) before milling the coarse flour in a speed rotor mill (Pulverisette 14, Fritsch).

## 2.3 | Analytical methods

### 2.3.1 | Degree of sprouting

There are eight degrees of sprouting (see Figure 1) which were determined by visually classifying the length of the coleoptile and radicles of 300 kernels. During sprouting every day, the average DoS was calculated as the sum of relative occurrence of the different classes (i):

$$\text{Average DoS} = \sum_{i=0}^7 i \cdot \text{occurrence\%}(\text{DoS}_i) \quad (1)$$

This was derived based on a set of 300 kernels. Each kernel is evaluated for its individual DoS.

In Figure 1, the DoS is given. Grains of Degree 0 do not show any radicle growth. Degree 1 characterizes grains with visible embryos (small white point) and not yet visible radicles or coleoptile. Degree 2 indicates grains already showing a developed embryo emerging from the seed coat. The grains of Degree 3 have radicles with a length less than half a grain length. Degree 4 is represented by grains with a radicle length between half and a full grain length. Degree 5 grains are characterized by radicles longer than a full grain and having a miniscule coleoptile. Grains of Degree 6 have coleoptiles longer than a full grain length. Degree 7 describes grains with coleoptiles which are at least twice as long as the grain.

### 2.3.2 | Sprouting losses

The weight and the moisture content of the sprouted grains were determined by using the moisture analyzer MA 35 (Sartorius). Based on these data the losses during the sprouting process were calculated:

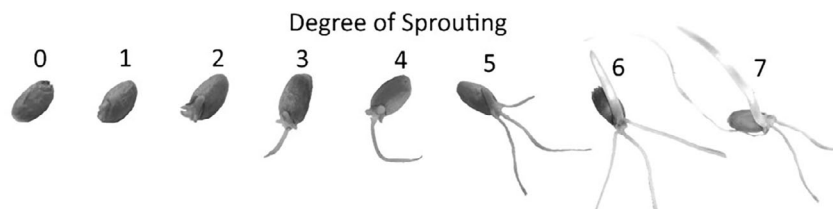
$$\text{Losses} = 1 - \frac{m(\text{dry matter based})_{\text{after sprouting}}}{m(\text{dry matter based})_{\text{native grains}}} \quad (2)$$

### 2.3.3 | Reducing sugar content

The determination of the reducing sugar content was described elsewhere (Krapf, Arysanto, et al., 2019). Average values and the SD were calculated from a twofold determination.

### 2.3.4 | α-amylase activity

The α-amylase activity was determined by using the Malt-amylase assay procedure (Megazyme International Ireland, 2015). Average values and the SD were calculated from a twofold determination.



**FIGURE 1** Definition of the degree of sprouting of wheat grains by the lengths of their coleoptile and radicles

### 2.3.5 | Viscous behavior of flour suspensions

The viscosity of an 10% (w/w) aqueous solution containing sprouted grain flour was determined using an Anton Paar rheometer MCR 302 equipped with a starch cell and a stirrer having a vane geometry. Throughout the experiments the stirrer speed was kept at 150 rpm. Copper(II) chloride at a concentration of 10 mM was added to the suspension in order to inactivate the enzymes, especially amylases according to Aquino, Jorge, Terenzi, and Polizeli (2003). The enzymes were inactivated to prevent further starch degradation during the heating cycle of the rheological measurements. Preliminary tests had shown that this precaution is necessary to determine the rheological properties reliably.

A 35 ml suspension was prepared at room temperature and stirred during the complete viscosity determination. During the determination the temperature of the sample was increased from 30 to 95°C with a heating rate of 1.5°C/min. Then, the suspension was stirred at a constant temperature of 95°C for 10 min before cooling the sample from 95 to 30°C with a cooling rate of 4.33°C/min. The temperature regime and heating rate were adopted from the method using an amylograph (DIN EN ISO 7973, 2016). The stirrer speed was chosen based on preliminary tests.

From these analyses the so-called *peak viscosity* (PV) was obtained. The average values and the SD were calculated from a two-fold determination.

### 2.3.6 | Ascorbic acid content

The ascorbic acid (vitamin C) content was determined according to the *Indophenol Method* (Nielsen, 2003). The indophenol solution was diluted eight times. The flour was dispersed in a mixture of 30 g/L metaphosphoric acid and 8% (v/v) acetic acid and centrifuged at 3,000g. A volumetric sample of the resulting supernatant was diluted and used for titration according to the method. The procedure was executed in duplicate per specimen.

### 2.3.7 | Extrudate properties

The cut extrudate strands were characterized using the so-called SEI, longitudinal expansion index (LEI), and hardness ( $F_{max}$ ). The sectional expansion rate is defined as the ratio of the circular cross-section area of the extrudates and the cross-section area of the die. It was determined based on the diameter of the extrudates. The given values are averages of six different extrudate strands from the same experimental

run. The dimensions of each strand were taken five times. The relative longitudinal expansion rate was derived from measuring the length of extrudates produced in 5 s.

The hardness of the extrudates was determined using a *Zwick testControl II* texture analyzer. A cylindrical probe with a diameter of 1 in. was used to break and crunch a single extrudate strand. The speed of the probe was 15 mm/min and the peak force to break an extrudate strand was defined as hardness of the extrudate. The given data points are the respective average of eight independent determinations.

The density was calculated assuming a cylindrical shape with an average diameter, average length, and mass of the extrudate strand. The average density was obtained from six different data sets of one extrudate.

The cold-water solubility index was determined by suspending 0.5 g of the milled extrudates (dry matter based) in 50 ml distilled water. The suspension was stirred for 30 min before the not dissolved material was filtered from the suspension (S&S 595) using a Büchner funnel. The solid residue was determined by weighing after removing the water from the filter cake by drying at 130°C for 1 hr. The cold-water solubility index in percent, which actually uses the ratio of the mass of dissolved extrudates and the mass of the solution multiplied by 100, equals the difference of the initial dry matter weight and the weight of the residue. Determinations were done in duplicate.

The total starch and total sugar content of the extrudates were analyzed by Medallion Labs (Minneapolis, MN) by use of the methods AOAC 977.20, AOAC 979.10 and AACC 76-11.

## 2.4 | Statistical evaluation (ANOVA)

The statistical evaluation of the properties studied after 3 days of sprouting was performed using Microsoft Excel 2016. The *p*-value (probability of error) was calculated by analyzing the experimental data by means of ANOVA (analysis of variance). A level of significance ( $\alpha$ ) of .05 was chosen.

## 3 | RESULTS AND DISCUSSION

### 3.1 | Effect of sprouting time and temperature on sprouted wheat flour properties

In a first step the effect of the five tested sprouting temperatures and three different sprouting periods was tested. In Figure 2, the average DoS and the underlying distribution within a sample are shown as a

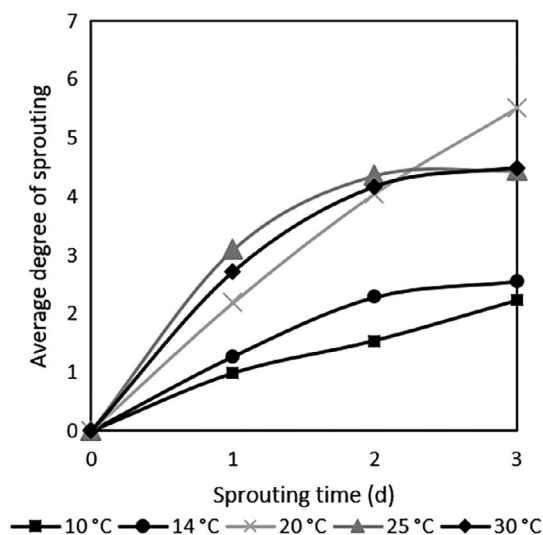
function of temperature after 3 days of sprouting. Furthermore, Figure 3 illustrates the development of the average degree over time for different sprouting temperatures.

One can clearly see an increase of the DoS over the sprouting period irrespective of temperature. Already after 1 day differences in sprouting progress between different temperatures were visible. Initially, the sprouting process at 25°C was most prominent. After 2 days the average DoS is practically indistinguishable for the temperatures of 20, 25, and 30°C. However, more detailed analysis revealed that the sample at 20°C showed a more homogeneous sprouting progression indicated by a lower *SD* of the DoS. In contrast to oat which showed almost no sprouting activity at 30°C (Krapf, Kandzia, et al., 2019), wheat sprouted really good at 20, 25, and 30°C. The highest average DoS was reached at 20°C after 3 days of sprouting. About 70% of the grains were found to have a DoS of 6, characterizing coleoptiles longer than a full grain length. The homogeneity of the grains DoS at 20°C was particularly high, indicating that large-scale production would be most robust at this temperature. In comparison, the sprouting process at 30°C exhibited a slightly lower average DoS after 3 days. This corresponds to the much lower homogeneity at 30°C, for example, 15% of the grains had DoS 7, 15% DoS 6, 25% DoS 5. After 3 days the average DoS was the lowest for the sprouting taking place at 10°C. Here about 50% of the grains had a DoS of 2 which indicates an already developed embryo emerging from the seed coat.

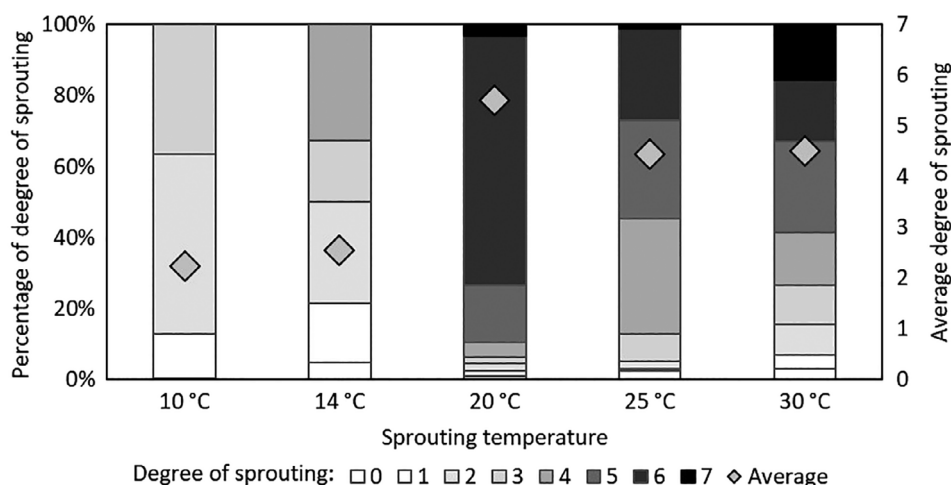
Growth and respiration processes are part of the sprouting process and affect the losses in dry matter. In Table 2 the losses during sprouting at different temperatures are listed. One can see that the losses due to sprouting were quite low after 3 days. Maximum losses found were 5% (w/w). These were found after 3 days at sprouting temperatures of 20 and 25°C. In general, the highest losses were detected for samples with the highest DoS and hence the biggest visual changes. Suhasini and Malleshi (1995) found malting losses of up to 15% for wheat sprouted for 3 days at temperatures between 20 and 30°C. However, these losses also involved the separation of the vegetative growth (coleoptile and radicles). In our study, the coleoptile and radicles were not separated and were part of the sprouted grain flour.

In Table 2, the respective values of these parameters are given after 3 days of sprouting. In addition, Table 1 presents the *p*-values of the properties considered in this study which were calculated as part of the ANOVA. The analysis reveals, that the calculated *p*-value is lower than the level of significance, indicating that stated differences are significant.

From the results in Table 2, it becomes clear that the chosen sprouting temperature has a substantial effect on the  $\alpha$ -amylase activity. In native grains a very low  $\alpha$ -amylase activity was found which indicates a new synthesis of  $\alpha$ -amylase throughout the sprouting process (Bewley, 2001). In Figure 4a, the  $\alpha$ -amylase activity is shown as function of the DoS. For low degrees of sprouting, also a low  $\alpha$ -amylase activity was found irrespective of the temperature. In Figure 3, it can be seen that the average DoS is the highest after 3 days at 20°C where it reached about 5.5. As seen in Figure 4a, this corresponds with the highest  $\alpha$ -amylase activity (140 U/g). This is in line with the findings of Reddy, Ching, and Metzger (1984) and Müller



**FIGURE 3** Average degree of sprouting as function of the sprouting time at various temperatures



**FIGURE 2** Effect of the temperature on the degree of sprouting after 3 days

	$\alpha$ -Amylase activity	Reducing sugar content	Peak viscosity	Vitamin C content
p-Value	1.3146E-11	3.448E-17	2.9042E-12	.02247834

**TABLE 1** *p*-Values from statistical analysis (ANOVA) of studied properties of sprouted wheat flour after 3 days of sprouting

**TABLE 2** Losses,  $\alpha$ -amylase activity, peak viscosity, reducing sugar, and ascorbic acid content in grains sprouted for 3 days at different temperatures

Sprouting temperature	Losses	$\alpha$ -Amylase activity	Reducing sugar content	Peak viscosity	Ascorbic acid content
	% (w/w)	U/g	g/100 g	Pa s	mg/100 g
Native	–	1 $\pm$ 0.00	2.91 $\pm$ 0.01	1.22 $\pm$ 0.00	0.97 $\pm$ 0.15
10°C	0.97	29 $\pm$ 0.51	6.03 $\pm$ 0.01	0.97 $\pm$ 0.00	4.95 $\pm$ 0.22
14°C	0.99	65 $\pm$ 0.58	10.56 $\pm$ 0.00	0.94 $\pm$ 0.00	6.72 $\pm$ 0.22
20°C	4.72	140 $\pm$ 0.16	16.84 $\pm$ 0.00	0.65 $\pm$ 0.00	8.35 $\pm$ 0.51
25°C	5.17	85 $\pm$ 0.00	15.91 $\pm$ 0.01	0.73 $\pm$ 0.00	7.24 $\pm$ 0.74
30°C	4.31	43 $\pm$ 0.44	24.99 $\pm$ 0.00	0.77 $\pm$ 0.00	7.61 $\pm$ 0.37
p-Value		1.3E-11	3.5-17	2.9E-12	.22

(2015). Correlating the average DoS (Figure 3) with the data for the  $\alpha$ -amylase activity (Figure 4a), it can be seen that after 3 days of sprouting at either 25 or 30°C an average DoS of only 4.5 was obtained with  $\alpha$ -amylase activities of 85 U/g and 43 U/g, respectively. This clearly shows a lower  $\alpha$ -amylase activity than at 20°C (140 U/g). After 3 days of wheat sprouting an average DoS of around 2.3 was found for temperatures of 10 and 14°C. One would hence expect for these lower degrees of sprouting an even lower  $\alpha$ -amylase activity than for the other investigated temperatures. That is true, a sprouting temperature of 10°C resulted in an  $\alpha$ -amylase activity of 29 U/g while 14°C resulted in 65 U/g. The temperature of 14°C is often used for malting in brewery applications (Jacob, 2016). The process is essentially designed to degrade the starch contained in malt with existing amylolytic enzymes in a wort to fermentable sugars. The  $\alpha$ -amylase activity found in this work is exceptionally high, 65 U/g. However, the conversion of starch to reducing sugars is more prominent at higher temperatures despite the  $\alpha$ -amylase activity.

Furthermore, it has to be pointed out that it is known that the extent of the enzyme synthesis differs for wheat cultivars (Reddy et al., 1984).

Due to the presence and, thus, the activity of  $\alpha$ -amylase during the sprouting process, starch is degraded into dextrins and short-chain sugars (Bewley, 2001). The so produced short-chain sugars exhibit a reducing potential and can be determined as part of the reducing sugar content which is used as a marker of the starch degradation in this study. In the given study, the reducing sugar concentration is related to the reducing content of maltose because maltose was used for the calibration. In Figure 4b, the content of the reducing sugars with respect to a maltose equivalent as function of the DoS is presented.

Combining the data in Figure 4b and Figure 3, it can be deduced that the reducing sugar content increases with increasing DoS, thus if considering the data from Figure 3, with increasing sprouting time. An exponential relationship was found and is also presented in Figure 4b

where the results for reducing sugar is plotted as a function of DoS disregarding the temperature the data was obtained at.

However, if only considering the data after 3 days of sprouting, the reducing sugar content increases with increasing sprouting temperature. The highest reducing sugar content was found after 3 days of sprouting at 30°C. The  $\alpha$ -amylase activity seems to be the highest at this temperature. According to Mohamed, Al-Malki, and Kumosani (2009), the temperature optimum of wheat malt  $\alpha$ -amylase is 50°C. This is no contradiction to the above studied  $\alpha$ -amylase activities which were measured at a specific temperature (40°C; Megazyme International Ireland, 2015) and consequently rather provide information about  $\alpha$ -amylase concentration during sprouting. It is conceivable that at 30°C a lower concentration of  $\alpha$ -amylase was synthesized, however, due to enhanced temperatures the enzymes were more active at this sprouting temperature in the grain and the starch was more efficiently mobilized and more reducing sugar were formed.

Furthermore, the reducing sugar content was described by an exponential trend line as function of the DoS. The correlation is rather good with  $R^2 = .88$  which indicates that the DoS concept can be used to estimate the sweetness of sprouted wheat products.

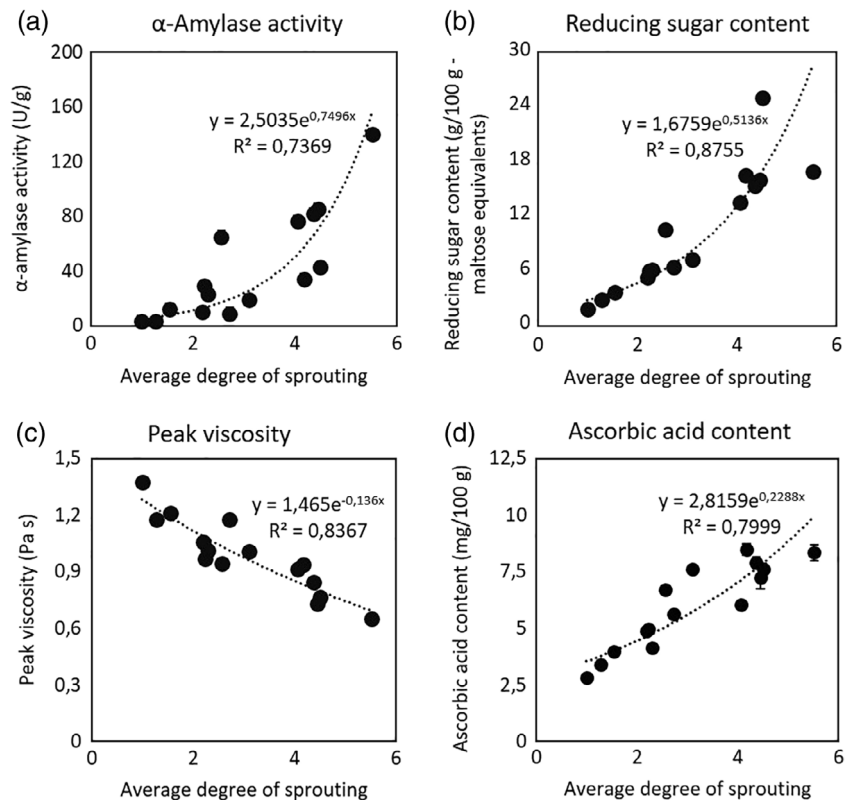
In Figure 4c, the peak viscosity is given as function of the average DoS. The peak viscosity decreases with increasing DoS. In light of the data depicted in Figure 3, it can be seen that as one would expect the lowest peak viscosity is found after 3 days of sprouting at 20°C, the highest after 1 day of sprouting at 10°C. The low peak viscosity after the sprouting process indicates a lower starch content and a higher degree of starch degradation whereby the swelling capacity of the starch granules was probably affected.

These results are in accordance with the decreased cooked paste viscosity due to increasing sprouting times found by Suhasini and Malleshi (1995).

In a previous study the dependence of the peak viscosity on the average molecular size of starch molecules was demonstrated (Krapf, Majocco, Walther, & Flöter, 2019). Due to a smaller average molecular



**FIGURE 4**  $\alpha$ -Amylase activity (a), reducing sugar content (b), peak viscosity (c), and ascorbic acid content (d) as function of the average degree of sprouting of all temperatures and sprouting times. Error bars of peak viscosity and reducing sugar content are too small to be identifiable. An exponential trend line was added and the function and coefficient of determination are given



size of starch, flour suspensions were found to have a lower viscosity and, thus, a higher flowability.

Moreover, the nutritional improvement of the wheat grains due to the variation of the sprouting conditions were studied. Because of its high reactivity (Lintschinger et al., 1997), ascorbic acid was chosen as a marker vitamin and its concentration was determined (see Figure 4d). In native wheat grains hardly any ascorbic acid was found. After 3 days of sprouting, the average DoS varied between 2.1 and 5.5. At all these varying degrees of sprouting a notable ascorbic acid content was detected. In general, the ascorbic acid content increases with increasing DoS. The data were described by an exponential trend with a respectable correlation between ascorbic acid content and degree of sprouting ( $R^2 = .8$ ).

The results are in accordance with Swieca and Dziki (2015) who found the highest antiradical activity at 20°C in comparison those found at 25°C.

Significant amounts of ascorbic acid are present to protect the growing grain from oxidation processes.

However, embryos of wheat were found to be free of ascorbic acid. In the first hours of germination the embryos are protected by ascorbic acid which has been formed by the reduction of already present low amounts of dehydroascorbic acid. With progressing sprouting, reducing sugars were formed, and converted into ascorbic acid (see also Figure 4b; Gara, Pinto, & Arrigoni, 1997).

Besides, ascorbic acid is of technological importance. It is used in bread and flour industry as a redox agent. Addition of only 10 ppm ascorbic acid improves the rheological properties of doughs. Further, it was found that ascorbic acid increases the gas retention capacity,

the dough strength, and the volume of biscuits (Grosch, 1986). The amounts of ascorbic acid formed during sprouting seem to be sufficient to achieve similar positive effects which would make further addition of ascorbic acid redundant.

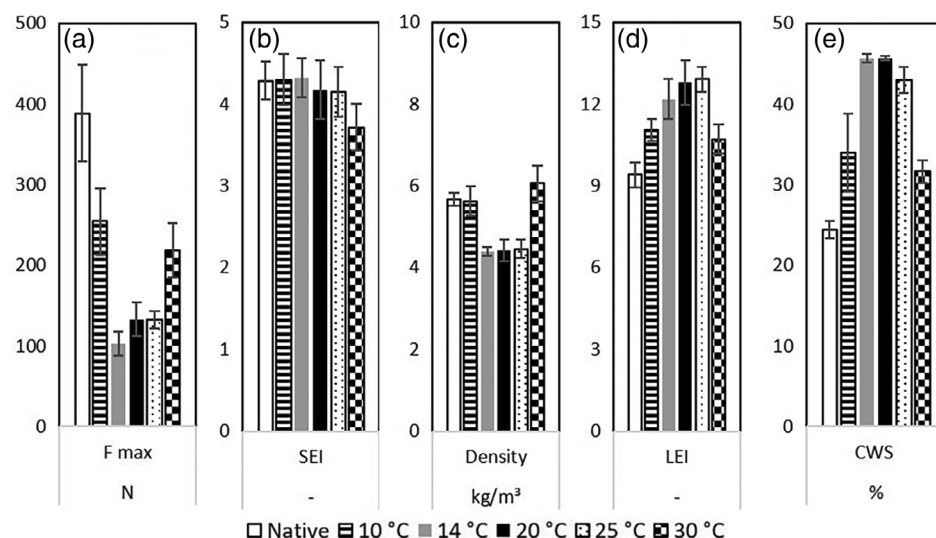
Concluding from the results described above a sprouting temperature of 20°C in combination with a duration of 3 days should be applied for the sprouting of wheat grains to obtain the most homogeneous sprouting, at the highest DoS, and with the formation of the highest amylase activity and vitamin C. At this temperature, a high amount of reducing sugars is formed and the lowest peak viscosity was analyzed. These findings can leverage and used for extrudate production.

### 3.2 | Sprouting temperature effect on extrudate properties

In Figure 5, key characteristics of extrudates as affected by the sprouting material used for their production are depicted.

Due to the use of sprouted grain flour for the production of extrudates, typical properties of the extrudates such as breaking force ( $F_{max}$ ), cold-water solubility (CWS), expansion (LEI and SEI), and density are affected.

As shown in Figure 5a, the hardness of the extrudates decreases when using sprouted grain flour. The samples which contain sprouted grain flour from grains which were sprouted at 14, 20, and 25°C were the easiest to break extrudates while the extrudates produced from grains sprouted at 10 and 30°C or from native grains show the highest



**FIGURE 5** Effect of sprouting on wheat flour and their extrudate properties: hardness  $F_{max}$  (a), sectional expansion index SEI (b), density (c), longitudinal expansion index LEI (d), cold-water solubility index CWS (e). The grains were sprouted at five different temperatures. CWS, cold-water solubility; LEI, longitudinal expansion index; SEI, sectional expansion index

**TABLE 3** Correlation of extrusion system parameters and product properties of wheat which was sprouted for 3 days at different temperatures; correlations greater  $\pm 0.85$  are highlighted bold

	DoS	$F_{max}$	SEI	Density	LEI	PV flour	CWS extrudate	Die pressure	Die temp	Total sugar content	Total starch content
DoS	1.00	<b>-0.88</b>	-0.40	-0.41	0.80	<b>-0.99</b>	0.75	<b>-0.96</b>	<b>-0.92</b>	<b>0.94</b>	<b>-0.90</b>
$F_{max}$		1.00	0.06	0.76	<b>-0.93</b>	<b>0.90</b>	<b>-0.96</b>	<b>0.86</b>	<b>0.96</b>	<b>-0.91</b>	0.81
SEI			1.00	-0.46	0.09	0.28	0.18	0.52	0.19	-0.38	0.58
Density				1.00	<b>-0.86</b>	0.46	<b>-0.90</b>	0.43	0.72	-0.53	0.40
LEI					1.00	-0.82	<b>0.96</b>	-0.79	<b>-0.96</b>	0.82	-0.72
PV flour						1.00	-0.79	<b>0.92</b>	<b>0.91</b>	<b>-0.92</b>	0.84
CWS extrudate							1.00	-0.72	<b>-0.91</b>	0.78	-0.65
Die pressure								1.00	<b>0.93</b>	-0.82	<b>0.86</b>
Die temp.									1.00	<b>-0.93</b>	<b>0.88</b>
Total sugar content										1.00	<b>-0.97</b>
Total starch content											1.00

Abbreviations: CWS, cold-water solubility; DoS, degree of sprouting; LEI, longitudinal expansion index; PV, peak viscosity; SEI, sectional expansion index.

hardness. The SEI, indicating radial expansion of the extruded mass after leaving the die and drying, is only slightly affected by the sprouting process and temperature. However, a slight decrease with sprouting temperature is observed (Figure 5b). The density of the extrudates produced from grains sprouted at 14, 20, and 25 °C is minimally lower than that of extrudates produced from native grains or grains sprouted at 10 and 30 °C (Figure 5c). This agrees with the findings concerning the hardness of the extrudates (Figure 5a). Extrudates with a higher density are harder to break.

In Figure 5d, the longitudinal expansion is depicted. Here an increase is observed for the extrudates produced from sprouted grains sprouted at 14, 20, and 25 °C. The LEI of the extrudates produced from native grains is clearly smaller.

It is interesting to see that the three samples sprouted at 14, 20, and 25 °C are the hardest, have lowest density, and have the highest LEI. This can be used and leveraged in product development and producing extruded puffs.

Similar behavior is found for the cold-water solubility index of the milled extrudates made from the native or sprouted grains (Figure 5e). The extrudates based on the grains sprouted at 14, 20, and 25 °C have the highest DoS and the highest  $\alpha$ -amylase activities, which has an impact on starch degradation and hence cold-water solubility. Higher level of short-chain sugars increases the water solubility of the milled extrudates. These samples might have the shortest bowl life (stability in milk), which should also be considered in developing products from sprouted grains.

The increase in the CWS and LEI and the decrease of the breaking force of extrudates based on sprouted wheat compared to native was also found by Singkhornart, Edou-ondo, and Ryu (2014). In their study wheat was used which was sprouted at 25 °C for 3 days and was compared to native wheat samples.

In Table 3, the correlation matrix for simple linear relation between the determined system parameters and extrudate properties is given. Very interesting correlations were detected which will be



highly valuable for product development, process scale up, and production of products made with sprouted wheat. All correlations greater  $\pm 0.85$  are highlighted bold and will be discussed.

It was found that the peak viscosity of flour suspensions from sprouted grains decreases with increasing average DoS ( $R = -.99$ ). Furthermore, the starch content decreases with increasing average DoS, so that the pressure in the extruder die decreases.

The pressure difference in the extruder die is the driving force for expansion (Fan, Mitchell, & Blanshard, 1996). Due to the decreased pressure and the changes in the expansion behavior, the hardness of the extrudates is affected and a strong correlation between the average DoS and hardness of extrudates is found. Furthermore, a good correlation between the longitudinal expansion of the extrudates and the hardness is determined. Extrudates with a high DoS and hence a high LEI show a lower hardness ( $R = -.93$ ). In addition, the density and LEI of the extrudates correlated well ( $R = -.86$ ).

The average DoS correlates well with the die temperature of the extruder ( $R = -.92$ ). As explained above, sprouted grains suspensions with a high DoS and hence a greater starch degradation are lower-viscous and less frictional heat is produced during the extrusion process (Fan et al., 1996). Consequently, the extruded mass is subjected to less energy dissipation and the mass is heated up less. Die temperatures remain hence lower when flour relating to higher DoS is processed.

Furthermore, it was found that the total sugar content correlates well with the average DoS, which was already explained above for the reducing sugar content (Figure 4b). The increase in the total sugar content lowers the glass transition temperature (Fan et al., 1996), and hence contributes to the reduction of the viscosity ( $R = -.92$ ) and the hardness of the extrudates ( $R = -.91$ ). Similar results were found by Barrett, Kaletunç, Rosenberg, and Breslauer (1995), who studied the effect of sucrose on extrusion characteristics.

The lowered viscosity in the extruder coming along with the increase in the total sugar level can also explain the good correlation between the die temperature and the total sugar content ( $R = -.93$ ).

Reasonably a good correlation between the total starch and sugar content was found ( $R = -.97$ ). The starch content is reduced at specific sprouting temperatures, at the same time the total sugar content increases.

Compared to another study of ours (Krapf, Arysanto, et al., 2019), where the effect of the sprouting time was investigated, the results presented here reveal a similar effect of advantageous sprouting temperatures and long sprouting times.

## 4 | CONCLUSION

The study of the effect of the sprouting temperature on wheat flour characteristics offered systematic results and new insights. Significant effects of the sprouting time and sprouting temperatures on flour and extrudate characteristics were identified.

This study suggests that the DoS concept which was presented in an earlier study (Krapf, Kandzia, et al., 2019), yields good correlation

with the sprouted wheat flour and extrudate properties as affected by the sprouting temperature. Building on this, it is conceivable that just by visual inspection and evaluation of the sprouted grains to predict respective product properties.

Generally, a sprouting temperature of approximately 20°C was found to result in the highest sprouting activity.

The highest sprouting activity corresponded well with the results of the studied extrudate property changes, whereby flour from sprouted wheat was used. Using wheat flour from grains sprouted at 20°C showed the greatest changes compared to extrudates based on native wheat. These extrudates were found to have the lowest hardness and density but also the highest longitudinal expansion and solubility.

Combining the results presented here with earlier work of ours (Krapf, Arysanto, et al., 2019) it appears that optimization of sprouting time at a given temperature or optimization of the sprouting temperature at a given time yield similar extrudate properties. The methodical investigation of this and previous studies on the sprouting process have resulted and very valuable insights that can be useful in developing products from sprouted grain. The results of this work will help making it easier to optimize product and process development.

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